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ASSOCIATION OF MARKER OF INFLAMMATION, HEPATIC ENZYMES AND LIPID PROFILE IN TYPE 2 DIABETES

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Abstract. *Type 2 diabetes mellitus (T2D) is a condition characterized by hyperglycemia as well as chronic inflammation, and is associated with disturbed lipids metabolism and impaired hepatic function. It is well known that the liver plays a key role in maintenance of normal glucose levels during the fasting and post prandial periods while C reactive protein as a marker of inflammation is produced in the liver. Altered lipoprotein levels and elevated hepatic enzymes have been identified as an independent risk factor for the development of many metabolic disorders including T2D. Aim of this study was to evaluate C-reactive protein (CRP) as well hepatic enzymes and to find their association with the lipid profile in non-treated T2D patients. Biochemical parameters, CRP, hepatic enzymes (alanine amino transferase, aspartate amino transferase, gamma-glutamyl transferase, and alkaline phosphatase) were measured by using VITROS 350 Chemistry System. Glycemic control parameters, lipid profile and liver enzymes were increased in diabetics and differed from control group ($p<0.001$). The significant association between CRP with HDL levels as well as association of ALT and GGT activity with HDL levels was observed in control group. Also, a negative association of GGT with VLDL levels was revealed in healthy subjects. In non-treated diabetics a negative significant association between AST and HDL levels as well as a positive association of AST and LDL levels was found while lack of association between lipid profile and other liver enzymes. Interestingly, in diabetes patients a negative association between CRP and AP levels was observed. These findings suggest that marker of inflammation (CRP), hepatic enzymes activities and impaired lipid metabolism may play an important role in pathogenesis of T2D and related complications.*

Keywords: *Inflammation, liver enzymes, lipid profile, type 2 diabetes*

1. INTRODUCTION

Type 2 diabetes mellitus (T2D) is a condition characterized by chronic hyperglycemia and low-grade inflammation associated with insulin resistance, dyslipidemia, and impaired liver function. It is well known that the liver plays an important role in maintaining normal glucose levels during fasting and in the postprandial period [1-2]. Although the pathogenesis is unclear, insulin resistance and chronic inflammation are thought to play an important role in triglyceride and free fatty acids (FFAs) accumulation in liver cells (Figure 1). Also, enhanced oxidative stress, lipid peroxidation, mitochondrial dysfunction contributes to the development of a wide spectrum of liver conditions especially non-alcoholic fatty liver disease (NAFLD) [3-5].

Previously studies have shown that the altered lipoprotein concentrations (total cholesterol, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol, very-low density lipoprotein cholesterol) and disturbed activity of liver enzymes: alanine amino transferase (ALT), aspartate amino transferase (AST), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP) have been identified as independent risk factors for the increased prevalence and incidence of fatty liver disease as well as T2D [6, 7].

Contradictory results have been observed in some of them, but changed and elevated serum activities ALT and GGT, and more recently increased ALP, together with increased CRP levels and triglycerides as well a very-low density lipoprotein cholesterol, were significantly associated with incidence of T2D and development of different conditions of fatty liver [8]. The underlying mechanisms remain uncertain but it is assumed that genetic predisposition, age, gender, ethnicity and race, overweight and low-physical activity represent the main risk factors for development both disease, fatty liver and diabetes [9, 10].

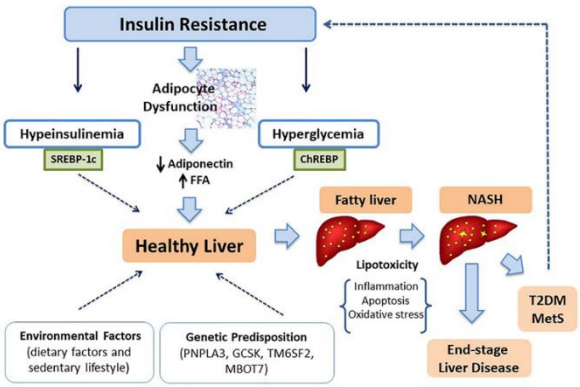

Figure 1. The role of insulin resistance in fatty liver and T2D development and progression.

The aim of this study was to evaluate C-reactive protein (CRP) as well as hepatic enzymes and find their

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association with lipid profile in newly diagnosed T2D patients.

2. Materials and methods

2.1. Subjects

A total of 51 subjects were enrolled in the study: 24 patients with newly diagnosed diabetes, recruited from Clinical Centre University of Sarajevo, and 27 healthy controls. Each participant gave written informed consent. The study was performed in accordance with the Declaration of Helsinki. T2D was diagnosed according to the American Diabetes Association from 2021 that includes fasting glucose ≥ 5.6 mmol/L, and glycated hemoglobin ≥ 6.5% [11]. Patients with an acute infection and/or inflammation, endocrine disorders, liver disease and those on insulin therapy were excluded from the study. A control group was comprised of 27 healthy, non-obese subjects, who were not taking any medication.

2.2. Biochemical analyses

Blood samples were drawn after an overnight fast, centrifuged at 3000 ×g for 10 min at 4 °C, and stored at −80 °C until the analyses The biochemical parameters including fasting glucose, HbA1c, CRP, lipid profile (total cholesterol, triglycerides, high density lipoprotein cholesterol), hepatic enzymes (alanine amino transferase-ALT, aspartate amino transferase-AST, gamma-glutamyl transferase-GGT, and alkaline phosphatase-ALP) were determined by standard procedures with an autoanalyzer VITROS 350 Chemistry System (Ortho-Clinical Diagnostics, Rochester, New York, USA). Glycated hemoglobin (HbA1c) was measured in the whole blood with EDTA by a NGSP-certified affinity separation (by cation exchange chromatography and affinity chromatography) method, using the BT 2000 plus chemical autoanalyzer (Biotecnica Instruments, Rome, Italy). Low density lipoprotein cholesterol (LDL-C) was estimated by Freidwald's formula [12].

2.3. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 17 software. Data are presented as the medians (interquartile range) for the continuous variables and non-normally distribution data. The differences in biochemical parameters between diabetics and controls were assessed by Mann-Whitney test, and Spearman's correlation analysis was used to test the correlation between inflammation and lipid markers with liver enzyme activities. In statistical analyses, a two-tailed p-value < 0.05 was considered statistically significant.

3. Results and discussion

The characteristics of newly diagnosed diabetic patients and the control group are shown in Table 1. Glycemic control parameters, i.e. fasting glucose and glycated hemoglobin levels, C-reactive protein, as well as lipid profile levels and liver enzymes activity, were higher in the diabetic group and there were significantly different compared to the control group (p<0.001).

Table 1. The clinical and biochemical characteristics of patients with the diabetes and control subjects.

Parameters	Control	Diabetes patients	*p**
Number	27	24	-
Gender (M/F)	14/13	14/10	-
Glucose, mmol/L	5.37 (5.24-5.49)	8.20 (7.59-8.81)	0.000
HbA1c, %	5.74 (5.57-5.90)	7.16 (6.88-7.44)	0.000
Serum TC, mmol/L	5.34 (5.02-5.65)	4.53 (4.12-4.94)	0.000
Serum HDL, mmol/L	1.27 (1.16-1.38)	1.17 (0.89-1.44)	0.000
Serum LDL, mmol/L	3.45 (3.11-3.78)	2.47 (2.08-2.86)	0.000
Serum VLDL, mmol/L	1.03 (0.50-1.55)	0.95 (0.80-1.10)	0.000
Serum TG, mmol/L	1.68 (1.28-2.08)	2.08 (1.77-2.40)	0.000
CRP, mg/L	2.07 (1.19-2.95)	3.29 (2.41-4.17)	0.000
ALT, U/L	17 (15-20)	30 (18-41)	0.000
AST, U/L	18 (16-20)	23 (14-32)	0.000
GGT, U/L	27 (23-32)	49 (18-80)	0.000
ALP, U/L	63 (56-69)	60 (52-67)	0.000

Values represent medians (lower–upper quartile). HbA1c-glycated hemoglobin; TC-total cholesterol; HDL-high density lipoprotein cholesterol; LDL-low density lipoprotein cholesterol; VLDL-very low density lipoprotein cholesterol; TG-triglyceride; CRP-C-reactive protein; ALT-alanine amino transferase; AST- aspartate amino transferase, GGT-gamma-glutamyl transferase; ALP-alkaline phosphatase. *Significance of difference in Mann-Whitney test.

The relationship between inflammation marker, C-reactive protein and liver enzymes: AST, ALT, GGT, and ALP with lipid profiles were explored. In the control group, CRP levels a positive correlated with HDL levels (p=0.010) (Table 2), while there were no significant associations between CRP levels and liver enzymes activity in healthy subjects (p>0.05). In the non-treated diabetics (Table 3), no significant association between CRP levels and lipids was observed (p>0.05). It was showed a trend of association between HDL levels and ALT as well GGT (p=0.004, and p=0.003 respectively) in healthy controls (Table 2). Also, a negative association with VLDL levels and GGT activity (p=0.017) was observed in the control group (Table 2). A negative association between AST and HDL levels (Table 3) as well as a positive association of AST and LDL levels were demonstrated in diabetics group/patients (p=0.044, and p=0.037, respectively) (Table 3). Interestingly, it is a negative significant association between CRP levels and ALP activity was showed in non-treated diabetes patients (p=0.037) (Table 3).

Inflammation is involved in obesity, dyslipidemia and IR, and is a risk factor for the development of T2D [1, 2, 13]. In this work, the effect of CRP as marker of inflammation and the level of the lipid profile was examined. A significant association of CRP levels with HDL was observed only in the control group (Table 2).

Table 2. Spearman's correlation coefficient (rho) between CRP levels with glucose, HbA1c, and serum lipid profile in controls and diabetic patients (total cholesterol, HDL, LDL, VLDL and triglyceride)

Parameters	Controls	Diabetics
	CRP	CRP
Glucose	r=0.047 p=0.816	r=-0.117 p=0.596
HbA1c	r= -0.001 p=0.995	r=-0.069 p=0.754
Total cholesterol	r= 0.147 p=0.466	r= 0.154 p=0.482
HDL-cholesterol	**r=0.487 p=0.010**	r=0.130 p=0.554
LDL-cholesterol	r=0.045 p=0.825	r=0.317 p=0.151
VLDL-cholesterol	r= -0.348 p=0.076	r=-0.288 p=0.193
Triglyceride	r=-0.249 p=0.210	r=-0.255 p=0.241

CRP- C reactive; protein; HbA1c-glycated hemoglobin, HDL-high density lipoprotein; LDL-low density lipoprotein; VLDL-very low density lipoprotein.

Significant association with CRP and lipid profile was not detected in the patients. The lack of association in this study might be attributed to the small sample size. On the other hand, a number of studies have explored the relationship of CRP with lipids level in diabetic patients, with conflicting results [14, 15].

Table 3. Spearman's correlation coefficient (rho) between CRP levels and serum lipid profile with liver enzymes activity (AST, ALT, GGT, and ALP) in controls.

Enzymes	AST	ALT	GGT	ALP
Glucose	r=-0.285 p=0.149	r=-0.126 p=0.531	r=-0.227 p=0.854	r=-0.268 p=0.206
HbA1c	r=-0.250 p=0.208	r= -0.085 p=0.672	r= 0.013 p=0.950	r=-0.165 p=0.441
CRP	r=-0.356 p=0.069	r= -0.024 p=0.906	r=0.273 p=0.168	r=0.270 p=0.202
Total cholesterol	r=-0.173 p=0.389	r= -0.002 p=0.992	r= -0.009 p=0.963	r= 0.320 p=0.127
HDL-cholesterol	r=0.319 p=0.104	**r=0.533 p=0.004**	**r=0.554 p=0.003**	r=0.184 p=0.390
LDL-cholesterol	r=-0.110 p=0.584	r=0.028 p=0.891	r= -0.086 p=0.671	r=-0.384 p=0.064
VLDL-cholesterol	r=-0.300 p=0.129	r= -0.325 p=0.098	**r= -0.458 p=0.017**	r=-0.304 p=0.148
Triglyceride	r=-0.290 p=0.142	r=-0.307 p=0.119	r=-0.358 p=0.067	r=-0.276 p=0.193

AST-aspartate aminotransferase; ALT-alanine aminotransferase; GGT-gamma glutamyl transpeptidase; ALP-alkaline phosphatase; HbA1c-glycated hemoglobin, CRP- C reactive protein; HDL-high density lipoprotein; LDL-low density lipoprotein; VLDL-very low density lipoprotein.

Also, possible explanation for these results are facts that a newly diagnosed patients with T2D and there were yet not disturbed lipid metabolism and lipid levels is in reference range/interval. Total cholesterol levels for cases were 4.53 mmol/L while normal range <5.2 mmol/L, HDL although lower than in controls, 1.17 mmol/L levels is in optimal value of 0.9-1.45 mmol/L for both gender, and also LDL levels of 2.47 mmol/L in optimal range of <2.49 mmol/L. Although TG values were higher in patients compared to healthy controls 2.08 mmol/L, and this lipid parameter was within the reference values of < 2.3 mmol/L. VLDL values were slightly elevated in diabetics, while in controls they were within the reference interval (0.1-1.7 mmol/L).

Table 4. Spearman's correlation coefficient (rho) between CRP levels and serum lipid with profile liver enzymes activity (AST, ALT, GGT, and ALP) in diabetes patients.

Enzymes	AST	ALT	GGT	ALP
Glucose	r=0.039 p=0.856	r=0.035 p=0.871	r=0.319 p=0.129	r=0.321 p=0.225
HbA1c	r=0.303 p=0.150	r=0.100 p=0.642	**r=-0.047 p=0.828**	r=0.050 p=0.855
CRP	r=0.108 p=0.625	r=-0.089 p=0.695	r=0.076 p=0.732	**r=-0.524 p=0.037**
Total cholesterol	r=0.250 p=0.239	r=0.139 p=0.516	r=0.285 p=0.178	r=0.232 p=0.387
HDL-cholesterol	**r=-0.414 p=0.021**	r=-0.289 p=0.171	r=0.013 p=0.953	r=-0.116 p=0.668
LDL-cholesterol	**r=0.438 p=0.037**	r=0.070 p=0.752	r=0.354 p=0.097	r=0.080 p=0.777
VLDL-cholesterol	r=-0.193 p=0.377	r=0.034 p=0.878	r= -0.208 p=0.342	r=0.222 p=0.426
Triglyceride	r=-0.133 p=0.536	r=0.030 p=0.890	r=-0.183 p=0.391	r=0.209 p=0.437

AST-aspartate aminotransferase; ALT-alanine aminotransferase; GGT-gamma glutamyl transpeptidase; ALP-alkaline phosphatase; HbA1c-glycated hemoglobin, CRP- C reactive protein; HDL-high density lipoprotein; LDL-low density lipoprotein; VLDL-very low density lipoprotein.

Insulin resistance and inflammation are the main metabolic factors that affect liver function and also cause various fatty liver conditions [2, 7]. In routine analysis, the serum or plasma concentrations of liver enzymes in patients with or without T2D represent good marker for liver function. The measurements are simple and non-invasive, unlike the biopsy or liver imagining of samples, especially in healthy subjects.

In the present study, a negative significant association of CRP level with liver enzymes was found in the group of diabetes for ALP activity (Table 3), while no association of these variables was found in the control group (Table 2).

In previous studies, ALP activity is elevated in the metabolic syndrome (MetS) and strong associated with plasma/serum CRP levels. MetS is associated with high prevalence of T2D in the world population, and both conditions are correlated with increased obesity, dyslipidemia and other metabolic traits [3, 6]. Patents with T2D, especially those with newly diagnosed diabetes and without any drug therapy, have elevated levels of CRP and ALP activity. Also, ALP is elevated in fatty liver disease such as NAFLD due to excessive accumulation of fat in the liver. The reported data suggest that fat deposition in the liver causes hepatic insulin resistance and contributes to the development of systemic insulin resistance and hyperinsulinemia. Also, increased ALP activity in serum/plasma may be a response to oxidative stress, disturbed physiological and antioxidant metabolism such as glutathione metabolism, non-enzymatic protein glycosylation and glucose autoxidation. Thus, elevated ALP may be a valuable potential marker of liver fat and hepatic IR; therefore, an increase in ALP activity represents an independent risk factor for development of T2D [16].

It is well known that lipotoxicity is associated with increased IR, hepatic glucose production and

gluconeogenesis and can lead to elevation of glucose levels and impaired insulin secretion in body [17, 18].

In the group of newly diagnosed diabetes patients of the present study a strong association between AST with HDL and LDL levels were found (Table 3). Numerous studies have shown controversial results of association of AST activity and incidence of T2D because it is present in other tissues mostly within the mitochondria and is the less specific marker of liver damage compared to, for example ALT and GGT activity [19, 20]. In diabetic group, a negative association between GGT and HbA1c was found (Table 3). This is not a surprise because these are newly diagnosed patients without drug treatment and therefore without glycemic control, and HbA1c is a marker of glycaemia [21]. This study is one of the few that investigated the association of liver enzymes and lipid profile. Future research should be conducted on a larger number of participants to confirm these results.

4. Conclusion

In this work, a significant association of HDL with CRP levels as well as an increased liver enzyme, ALT and GGT activity in control subjects was observed. Also, in healthy subjects a negative association between GGT and VLDL was detected. The significant association between HDL and LDL levels with AST activity, and a strong negative association ALP with CRP levels were detected in diabetic patients.

These results suggest that marker of inflammation (CRP), hepatic enzymes activities and impaired lipid metabolism may play an important role in pathogenesis of T2D and related complications.

References

1. V. M. G. Regufe, C. M. C. B. Pinto, P. M. V. H. C. Perez, "Metabolic syndrome in type 2 diabetic patients: a review of current evidence," *Porto Biomed. J.*, vol. 5, no. 6, article no. e101, Nov/Dec. 2020. https://doi.org/10.1097/j.pbj.0000000000000101
2. H. Jeong et al., "C reactive protein level as a marker for dyslipidaemia, diabetes and metabolic syndrome: results from the Korea National Health and Nutrition Examination Survey," *BMJ Open*, vol. 9, no. 8, article no. e029861, Aug. 2019. https://doi.org/10.1136/bmjopen-2019-029861
3. N.H. Cho et al., "Abnormal liver function test predicts type 2 diabetes: a community-based prospective study," *Diabetes Care*, vol. 30, no. 10, pp. 2566-2568, Oct. 2007. https://doi.org/10.2337/dc07-0106
4. Š. Mandal, "Free fatty acids and hepatic activity in Type 2 diabetes," *RAD Conf. Proc.*, vol. 4, Virtual Conf., 2020, pp. 90–94. http://doi.org/10.21175/RadProc.2020.19
5. B. Filipovic et al., "The new therapeutic approaches in the treatment of non-alcoholic fatty liver disease," *Int. J. Mol. Sci.*, vol. 22, no. 24, article no. 13219, Dec. 2021. https://doi.org/10.3390/ijms222413219
6. Y. L. Wang, W. P. Koh, J. M. Yuan, A. Pan, "Association between liver enzymes and incident type 2 diabetes in Singapore Chinese men and women," *BMJ Open Diabetes Research and Care*, vol. 4, no. 1, article no. e000296, Sep. 2016. http://doi.org/10.1136/bmjdrc-2016-000296
7. L. Guan et al., "Prevalence and risk factors of metabolic-associated fatty liver disease during 2014–2018 from three cities of Liaoning Province: an epidemiological survey," *BMJ Open*, vol. 12, article no. e047588, Feb. 2022. http://doi.org/10.1136/bmjopen-2020-047588
8. A.-L. Kurniawan et al., "Association of two indices of insulin resistance marker with abnormal liver function tests: a cross-sectional population study in Taiwanese adults," *Medicina*, vol. 58, no. 1, article no. 4, Dec. 2021. https://doi.org/10.3390/medicina58010004
9. Y. Li et al., "Serum alanine transaminase levels predict type 2 diabetes risk among a middle-aged and elderly Chinese population," *Annals of Hepatology*, vol. 18, no. 2, pp. 298–303, Mar.-Apr. 2019. https://doi.org/10.1016/j.aohep.2017.02.001
10. S. Scapaticci, E. D'Adamo, A. Mohn, F. Chiarelli, C. Giannini, "Non-alcoholic fatty liver disease in obese youth with insulin resistance and type 2 diabetes," *Front. Endocrinol.*, vol. 12, article no. 639548, Apr. 2021. https://doi.org/10.3389/fendo.2021.639548
11. American Diabetes Association, "Classification and diagnosis of diabetes: Standards of medical care in diabetes—2021," *Diabetes Care*, vol. 44, Suppl. 1, pp. S15–S33, Jan. 2021. https://doi.org/10.2337/dc21-S002
12. W. T. Friedewald, R. I. Levy, D. S. Fredrickson, "Estimation of the concentration of low-density lipoprotein cholesterol in plasma," *Clin. Chem.*, vol. 18, no. 6, pp. 499–502, Jun. 1972. https://doi.org/10.1093/clinchem/18.6.499
13. Š. Mandal, A. Čaušević, "The correlation between C-reactive protein and regulation of glycemia in Type-2 diabetic patients," *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina*, vol. 48, pp. 5–8, Jun. 2017. Retrieved from: http://hemija.pmf.unsa.ba/glasnik/files/Issue%2048%20new/5-5-8-Mandal.pdf Retrieved on: Nov. 29, 2022
14. S. Ebtehaj, E. G. Gruppen, M. Parvizi, U. J. F. Tietge, R. P. F. Dullaart, "The anti-inflammatory function of HDL is impaired in type 2 diabetes: role of hyperglycemia, paraoxonase-1 and low grade inflammation," *Cardiovasc. Diabetol.*, vol. 16, article no. 132, Oct. 2017. https://doi.org/10.1186/s12933-017-0613-8
15. W.-T. Hsu et al., "Investigation of non-HDL cholesterol and C-reactive protein in diabetes patients," *Biomarkers and Genomic Medicine*, vol. 5, no. 3, pp. 107–109, Sep. 2013. https://doi.org/10.1016/j.bgm.2013.08.002
16. M. Webber, A. Krishnan, N. G. Thomas, B. M. Cheung, "Association between serum alkaline phosphatase and C-reactive protein in the United States National Health and Nutrition Examination Survey 2005-2006," *Clin. Chem. Lab. Med.*, vol. 48, no. 2, pp. 167–173, Feb. 2010. https://doi.org/10.1515/CCLM.2010.052
17. Y. Xing, J. Chen, J. Liu, H. Ma, "Associations between GGT/HDL and MAFLD: A cross-sectional study," *Diabetes Metab. Syndr. Obes.*, vol. 15, pp. 383–394, Feb. 2022. https://doi.org/10.2147/DMSO.S342505
18. G. Feng, L. Feng, Y. Zhao, "Association between ratio of γ-glutamyl transpeptidase to high-density lipoprotein cholesterol and prevalence of nonalcoholic fatty liver disease and metabolic syndrome: A cross-sectional study," *Ann. Transl. Med.*, vol. 8, no. 10, article no. 634, May 2020. http://doi.org/10.21037/atm-19-4516
19. Z. Zhu et al., "Associations of lipid parameters with non-alcoholic fatty liver disease in type 2 diabetic patients according to obesity status and metabolic goal

achievement," *Front. Endocrinol.*, vol. 13, article no. 1002099, Sep. 2022. https://doi.org/10.3389/fendo.2022.1002099

20. S. K. Kunutsor, A. Abbasi, T. A. Apekey, "Aspartate aminotransferase – risk marker for type-2 diabetes mellitus or red herring?," *Front. Endocrinol.*, vol. 5, article no. 189, Nov. 2014. https://doi.org/10.3389/fendo.2014.00189
21. J. Y. Wan, L. Z. Yang, "Liver enzymes are associated with hyperglycemia in diabetes: A three-year retrospective study," *Diabetes Metab. Syndr. Obes.*, vol. 15, pp. 545–555, Feb. 2022. https://doi.org/10.2147/DMSO.S350426